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Use of Cost Effective Semi-Automated (Mannual/Micro) MGIT System over BACTEC 960 to Perform First Line Anti-Tuberculosis Drugs Sensitivity Testing

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Abstract

Introduction: Multi-drug resistant tuberculosis (MDR-TB) that is the tuberculosis that is resistant to at least 2 of the first line anti-tuberculosis drugs is fatal infectious disease. Cases of MDR-TB are now increasing with 30,000 cases of MDR-TB reported in 2013 by national TB programme. Rapid diagnosis of MDR-TB is extremely important for rapid treatment of patient and to prevent spread of MDR-TB to other. BACTEC 960 system helps in rapid diagnosis but purchase of expensive instrument for the same is the limitation. However, the same purpose can be solved by use of semi-automated MGIT system. **Aims and Objectives:** Aim of this study is to do drug sensitivity testing of the first line anti-tuberculosis drugs with the use of semi-automated MGIT systems. 350 newly registered and suspected cases of tuberculosis in tertiary care hospital were included. Samples were processed for digestion and decontamination and inoculated in MGIT tubes and also on LJ medium. Reading was taken using semi-automated MGIT system. Positive tubes were confirmed by rapid test for *M. tuberculosis* and then drug sensitivity was performed. **Result:** Out of 350 samples, 62% were sputum; 33% were pleural fluid and rest 5% were lymph node, Ascetic fluid, CSF, pus. Average day of positivity by MGIT was 13 - 20 days as compared to 25 - 37 days by solid medium, which was statistically significant with p value < 0.01. MDR cases were 2% out of 350 samples. **Conclusion:** Manual MGIT System is a simple, efficient, safe to use diagnostic system. It does not require any expensive/special instrumentation other than the UV lamp for detection of fluorescence. The rapidity by which mycobacteria are detected is the most important advantage of the Manual MGIT. In areas with limited resources where purchase of expensive instruments such as the MGIT960 is out of scope, the use of manual MGIT for rapid susceptibility testing for MDR-TB could be a possibility.

Keywords

Semi-Automated MGIT System, MDR-TB, First-Line Anti-Tuberculosis Drugs

1. Introduction

Tuberculosis is wide-spread and in many cases fatal infectious disease. India is the country with the highest burden of tuberculosis. Multi-drug resistant tuberculosis, which is resistant to Isoniazid and Rifampicin that are 2 first line anti-TB drugs, is increasing in many countries. Among TB patients reported by National TB programme in 2013, there were an estimated 300,000 cases of MDR-TB [1]. More than half of cases were in India, China and Russian federation. If these cases are not treated properly, they can spread resistant to other peoples and also develop resistant to other drugs like fluroquinolones and injectable amino glycosides, defined as extensive drug resistant. So for better management of drug resistant cases, early detection of resistant is extremely important so that effective treatment can be prescribed rapidly.

Drug resistant can be detected using gene-Xpert, Line probe assay, solid culture and liquid culture. Gen-Xpert detects give results within 2 hours and it can be performed directly from sample but it detects only resistant to Rifampicin [2]. Line probe assay detects the resistant gene for Isoniazid and Rifampicin and gives results within 24 - 48 hours but it can be used only on high grade smear positive sputum samples or for positive cultures only and is costly [3]. Drug sensitivity by solid medium can detect resistant for all first line drugs and is cost-effective but it takes longer time to obtain growth of mycobacteria and to perform drug sensitivity testing. MGIT tube which contains modified middle brook 7H9 broth in a tube with a fluorescence at the bottom of tube, emits its fluorescences when oxygen is consumed by an organism. These fluorescences can be detected by BACTEC MGIT 960 system [4] [5] [6]. However, purchase of expensive instrument such as MGIT 960 system is out of scope in countries like India. Semi-automated MGIT system which is based on the same principle and which does not need expensive instrument can be used for the same purpose. This study was performed to know the drug sensitivity of the first line drugs using semi-automated MGIT system.

2. Material & Methods

This study was a cross-sectional study, which was started after ethical clearance for the same. 350 newly registered and suspected cases of tuberculosis in tertiary care hospital were included in the study. Patients with major diseases like HIV, malignancy, patients on immunosuppressive therapy, immune compromised patients were excluded. Also patients with present or past history of anti-tuberculosis treatment were excluded. Samples were collected after giving brief information and after taking written consent. For pulmonary tuberculosis, sputum sample was collected along with oral instructions for proper sample collection like collection of sample in open air, before brushing, with

simple mouth gargling, taking inhalation for 2 - 3 times to obtain cough deeply from chest and spitting into container directly. For indoor patients with suspected of extra pulmonary tuberculosis, representative samples like pleural fluid, ascetic fluid, cerebrospinal fluid, pus, lymph node, tissue biopsy were collected by treating physician. For all samples, decontamination and concentration was done using 4% NAOH-2.9% tri sodium citrate (Petroff's method). Smear microscopy was done using ZN stain for all samples both before and after processing of samples. All processed samples were inoculated in liquid medium-MGIT tubes and also in solid medium-LJ medium to compare the growth. MGIT tubes with 7 ml of middle brook 7H9 medium was used into which 0.8 ml of PANTA was added to prevent contamination and to enhance growth and 0.5 ml of processed sample was added. Tubes were then incubated at 37˚C for 42 days. Reading was taken daily in first 3 weeks and then once a week for next 3 weeks using manual MGIT system. Tubes with granular turbidity or positive by semi-automated reader were inoculated on brain heart infusion broth to check for contamination and also smear is prepared for ZN stain and gram stain to check for acid fast bacilli and contamination respectively. If acid fast bacilli are seen by ZN stain and no contamination is there, then it was confirm by rapid test to differentiate M. Tb and NTM. If contamination is present, re-decontamination was done. Tubes which were negative after 42 days were declared negative. For LJ culture, 0.1 - 0.2 ml of processed sample was inoculated and incubated at 37˚C for 6 - 8 weeks.

Drug sensitivity testing was done using 1% proportional methods. Drugs are available as SIRE kit. Drugs were diluted with sterile distilled water to obtain the critical concentration of 1.0 μg/ml for Streptomycin, 0.1 μg/ml for Isoniazid, 1.0 μg/ml of Rifampicin and 5.0 μg/ml of Ethambutol. For Pyrazinamide, separate PZA medium tube containing 7 ml of broth was used. MGIT tubes positive for *M. tuberculosis* were labeled as day-0 on the day of its positive reading by reader, which were incubated for 1 more day before performing DST. If DST was done on day 1 or 2 old tubes, undiluted broth from positive tubes was taken for inoculation. For day 3, 4, 5 tubes, dilution of 1:5 is done using 4.0 ml of sterile saline and it is used as inoculum. Because tubes with day 6 or older are not used for DST and subculture from which inoculum was taken. Forone-sample5 MGIT tubes were labelled, one for GC (growth control without drug) and other 4were for Isoniazid, Rifampicin, Ethambutol, and Streptomycin. Then0.8 ml of SIRE supplement was added in all tubes and 0.1ml of reconstituted drugs were added in respective tubes as per labeled for drug. Then 500 μl of inoculums was added. A growth control tube (GC) was kept after doing 1:100 dilution. These whole set was incubated at 37˚C till growth control tube show growth. Because Pyrazinamide drug sensitivity test inoculums was diluted as1:10 instead of 1:100 dilution for growth control tube and special Pyrazinamide MGIT tubes and supplements were used. Reading of drug sensitivity was done using semi-automated MGIT reader daily. When growth in GC tube will be present readings will be taken on that day for drug tubes. If growth is present in drug containing tube then it will suggest resistance to that drug and if no growth in drug containing tube suggests susceptibility to that drug.

3. Result

Out of 350 samples, 62% were sputum; 33% were pleural fluid and rest 5% were lymph node, ascetic fluid, pus, pericardiac fluid, CSF samples. Male to female ratio of 350 samples was 2.7:1. Suspected cases were maximum in age group of 21 - 40. Out of 350 samples 59 samples (17%) were positive, out of which 48 (73%) were *Mycobacterium tuberculosis* (MTB) and 11 (19%) were *Non tuberculosis mycobacteria* (NTM). All 48 samples were positive by MGIT medium and 46 were positive by LJ medium. Average day of positivity by MGIT liquid culture method was 13 - 20 days while by LJ culture method it was 25 - 37 days. When results of ZN Microscopy of samples were compared with the day of getting a positive culture by solid and liquid culture method, it shows that as the smear grades increases, no. of days to get culture positivity decreases as shown in Table 1, which is statistically significant for MGIT liquid culture with p value less than 0.01 by using chi square test.

As in Table 2 and Table 3 MDR (multi drug resistant) isolates that are resistant to Isoniazid and Rifampicinis 2% (1isolate).

4. Discussion

The aim of this study was to do antibiotic sensitivity test of first line anti-tuberculosis drugs in all newly registered suspected patients of tuberculosis in tertiary care Hospital with the use of liquid medium MGIT (Mycobacterial growth indicator tube) using the manual MGIT reader. In present study all samples were cultured in MGIT liquid me-

Table 1. Comparison of grading of smear and its relation to culture positivity.

Smear grading by AFB direct smear	No. of samples	Average days of positivity by MGIT and LJ Medium Average day of positivity	
		By MGIT (average days)	By LJ media (average days)
Negative	11	20 days	37 days
Scanty	9	16 days	32 days
1	11	14 days	30 days
2	12	13 days	26 days
3	5	13 days	25 days

Table 2. Number of isolates showing resistant.

Resistance pattern	Number of isolates showing resistant (out of 48) samples)
Isoniazide + Rifampicin	1
Isoniazide alone	1
Rifampicin alone	0
Streptomycin alone	1
Isoniazide + streptomycin	1

Table 3. Number of isolates with different types of resistant pattern to first line anti-tuberculosis drugs with detected by Mannual MGIT system.

Drugs	Resistant (out of 48 samples)	Percentage (%)
Mono-resistant		
Isoniazid	1	2
Rifampicin	0	0
Pyrazinamide	0	0
Ethambutol	0	0
Streptomycin	1	2
Two drug resistant (MDR)		
Isoniazid + Rifampicin	1	2
Isoniazid + streptomycin	1	2

dium to obtain the rapid culture positivity of sample and in Lowenstein Jenson (LJ) medium for differentiation of *M. tuberculosis complex* from *Non tuberculosis mycobacteria.* In MGIT tubes on average, cultures were positive between 12 - 20 days, while in LJ medium were between positive 25 - 37 days which suggest that liquid culture are rapid to isolate the bacilli in comparison to solid culture. In a comparative study on 500 samples using the MGIT (960), L-J and direct smear, S. Rishi, Sinhaand Malhotra, in India, the reported recovery rate of mycobacteria was 98.06% for the MGIT from pulmonary and extrapulmonary samples in comparison to the L-J medium that showed are recovery rate of 63.95% [4]. Another study done in India using the MGIT960, by C Rodrigues *et al.* in 2009 also reported a 99.0% recovery rate with MGIT and 67.0% by L-J [5]. This study also demonstrated that rate of culture positivity was also depended on bacterial load/smear grading. In samples with microscopy result of 3+, MGIT required an average of 13 days and for negative samples it require average of 20 days which was statistically significant ($p < 0.001$). However a study done by Somoskvi *et al.* shows that MGIT required 12.6 days for smear positive sample and 15.8 days for smear negative results [6]. The study by Pfyffer *et al.* report had shown an average of 9.9 days for culture positivity [7]. Based on result of present study, detection and final identification of most cultures was rapid and reliable when manual MGIT liquid medium is used, which can helps in accurate diagnosis and administration of appropriate therapy to patients specially smear negative patients, although chances of culture contamination are more with liquid medium than solid/L-J method (5.5% - 15%). To prevent contamination, all sterile precaution should be taken like sterile work like adding PANTA-OADC supplement should be done before other processing are done, use individual falcan tubes for sample for adding NAOH-sodium citrate or phosphate buffer saline, cleaning of tubes outer surface before any processing in the tubes, using freshly prepared reagents, by following proper decontamination processing steps, using proper disinfectant for surface disinfectant of biosafety cabinet, using clean and autoclaved glass wares. For effective treatment and prevention of drug resistance, it depend sup on

the prompt availability of drug sensitivity results. Among the various diagnostic systems available for detection of drug resistance, the manual MGIT is rapid and sensitive and is validated by many previous study [8] [9] [10] [11]. DST for first line drugs has been thoroughly studied for them annual MGIT. Drug sensitivity testing of first line anti- tuberculosis drug was performed using liquid culture MGIT, results of which were available between 8 - 10 days after keeping drug sensitivity. Out of 48 samples 4 strains had shown resistance, of which one strain had shown resistant to Isoniazid and Rifampicin, one strain had shown resistant to Isoniazid and Streptomycin, one with resistant to Streptomycin al one and Isoniazid alone. In our study mono resistance in Isoniazid, Ethambutol and Streptomycin was 2.08% and no Rifampicin mono resistance was detected. Surveys done by the WHO [12] showed that the levels of primary resistance to INH ranged from 0% - 16.8% and for streptomycin 0.1% - 23.5%. Higher rates of resistance are reported from Kenya, India and Haiti while low levels were reported from England, Melbourne. Acquired drug resistance was higher than those of the primary resistance. For INH acquired resistance ranged from 4 - 53.3%, streptomycin 0 - 19.4%, rifampicin from 0 - 14.5% and ethambull 0 - 13.7%. Initial resistance to rifampicin ranged from 0 - 3% and the rate of ethambul resistance was 0 - 4.2% globally [13]. In India the initial drug resistance to INH is 18% - 20%, 4.8% - 14% for streptomycin. In the early 1990s, retrospective study done at New Delhi showed a high level of primary drug resistance to isoniazid (18.5%) and a low level of rifampicin resistance [14]. Data from India on acquired resistance to the antituberculosis drugs showed that any resistance to INH was in between 47.7% - 87.1%; for rifampicin it was 28.3 - 80.6. Prevalence of MDR-TB isolates among new cases in India [15] is shown in **Table 4**.

Table 4. Prevalence of MDR_TB among new cases of pulmonary TB in India.

Location	Period of study	NO. of isolates	MDR_TB (%)
Banglore	1980	436	1.1
Wardha	1982-1989	323	5.3
North Arcot	1985-1989	2779	1.6
Pondicherry	1985-1991	1841	0.8
Kolar	1987-1989	292	3.4
Jaipur	1989-1991	1009	0.9
New Delhi	1990-1991	324	0.6
Pune	1992-1993	473	1.0
Tamil Nadu	1997	384	3.4
North Arcot	1999	282	2.8
Luchknow	2000-2002	318	13.2
Hyderabad	2001-2003	714	0.14
Ernakulam	2004	305	2.0
Gujarat	-	1571	2.4
Mumbai	2004-2007	493	24

4. Conclusion

Manual MGIT System is a simple, efficient, safe to use diagnostic system. It does not require any expensive/special instrumentation other than the UV lamp for detection of fluorescence. The rapidity by which mycobacteria are detected is the most important advantage of the Manual MGIT. The time to detection of mycobacteria was 12 days and 21 days for the positive smear and negative smear specimen respectively. In areas with limited resources where purchase of expensive instruments such as the MGIT960 is out of scope, the use of manual MGIT for rapid susceptibility testing for MDR-TB could be a possibility. Susceptibility test results are obtained within a week of detection of mycobacteria. This would allow the early reporting of results to the clinicians thus helping in the rapid adjustment of the initial treatment of the patients. This would benefit not only the individual patients but the community as a whole in controlling the disease and its transmission in the society.

5. Limitation

Although liquid culture is rapid, it is not possible to see colony morphology and colony characteristic by using liquid culture. If mixed growth of *M. tuberculosis* with non-tuberculous mycobacteria occurs, it is not possible to isolate by using liquid culture.

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